Mammalian Melanin Formation: II. Histochemical Studies.

THOMAS B. FITZPATRICK,
Fellow in Dermatology and Syphilology, Mayo Foundation, Rochester, Minn.
&
A. BUNSEN LERNER,
University Hospital, University of Michigan, Ann Arbor, Mich.

As early as 1901 von Fürth advanced the hypothesis that melanin formation is the result of the action of an intracellular oxidase on aromatic or chromogen groups in certain protein molecules. Bloch, a Swiss dermatologist, stimulated by this hypothesis, attempted to prove it by experimental methods. He selected the naturally occurring amino acid, dihydroxyphenyl-L-alanine (dopa), as the substrate for his histochemical studies.

Bloch immersed frozen-fixed sections of human skin in a 1:1,000 solution of dopa buffered to pH 7.3, and noted that after twenty-four hours at room temperature black (melanin) granules were deposited in the cytoplasm of cells located in the basal layer of the epidermis. These specialized cells, which he called " melanoblasts," are located at the epidermal-dermal junction, and were considered by Bloch to be the site of melanin formation. Further evidence was provided by Bloch and his co-workers to support the hypothesis that the melanoblasts contained an enzyme, dopa oxidase, which catalyzed the oxidation of dopa to melanin within the cell. The dopa oxidase hypothesis of human melanin formation as advanced by Bloch never has been generally accepted as the explanation for the chemical mechanism underlying melanin formation. The main criticism has been that dopa never has been demonstrated in mammalian tissue.

In the past eight years it has been firmly established that extracts from mouse, horse and human melanomas contain tyrosinase and dopa oxidase activities (1). Furthermore, it has been shown that under certain conditions no true distinction can be made between tyrosinase and dopa oxidase activities in mammalian tissue. At the time Bloch carried out his important histochemical studies, little was known about the optimal conditions for the enzymatic oxidation of tyrosine. This may account for the fact that Bloch, working with mammalian tissue slices, obtained melanin formation from dopa but not from tyrosine.

In some recent histochemical experiments the authors, in collaboration with S. William Becker, Jr., demonstrated the formation of melanin from tyrosine in human white skin which had been irradiated with ultraviolet radiant energy for seven days before excision. Tissue slices cut from the biopsy material were incubated in tyrosine solutions at pH 7.1 for twenty-four to forty-eight hours. In paraffin sections of this material, there were seen large dendritic melanoblasts containing melanin granules in their cytoplasm, identical in their morphology to the "dopa-positive" cells obtained by Bloch. The catalytic effect of these cells on the oxidation of tyrosine to melanin is absent when the tissue slices are heated for ten minutes at 100°C. or when the tissue slices are incubated with sodium diethyldithiocarbamate, a copper-binding compound, for 6 hours prior to incubation in tyrosine. Since tyrosine, in contrast to dopa, which readily auto-oxidizes, is a stable amino acid which does not oxidize spontaneously to melanin *in vitro*, it is likely that the melanoblasts of human skin contain an intracellular oxidase, *tyrosinase*, similar to the enzyme described previously. The enzyme apparently exists in human skin in a partially inhibited state, and can be activated by ultraviolet radiant energy. The mechanism of this activation is not fully understood, but the inactivation of epidermal sulfhydryl by the ionizing radiation appears to play an important part. The data offer strong evidence that the pigment precursor in human melanin formation is tyrosine and not dopa.

The elaboration of melanin pigment in the epidermal melanoblast ordinarily depends on the available concentration of three substances:

1) The enzyme *tyrosinase:* a copper-protein complex attached to particles in the cytoplasm of the melanoblast.
2) A suitable substrate: usually tyrosine or dopa.
3) Molecular oxygen.

If any of these substances is absent, the formation of melanin is impaired.

The reaction of the three basic substances, tyrosinase, substrate and molecular oxygen, is controlled by several physico-chemical factors which determine the rate of melanin formation:

1) A catalytic substance, usually dopa, which can accelerate the tyrosine-tyrosinase reaction.
2) Chemical groups which normally inhibit copper enzymes, for example, sulfhydryl groups, normally found in the epidermis.
3) Physical and chemical factors, such as temperature, hydrogen ion concentration and oxidation-reduction potentials.

The quantity of melanin produced by the cell depends on the overall balance of these different forces.

REFERENCE.

1. LERNER, A. B. and FITZPATRICK, T. B. Biochemistry of Melanin Formation. *Physiol. Rev.*, 30:91-126, Jan., 1950.